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Remarks

Claims 24-33 and 41-49 are pending. A list of the pending claims is attached as an appendix for the Examiner's convenience. Support for amendments to claims 24 and 25 is at page 28, lines 17-30. New claim 50 is based on allowed claim 2 of U.S. Patent No. 5,330,754, a parent of the present application. Claim 50 is supported by Examples 1 and 2.

Specification

The Examiner suggests a change to the specification at page 11 because the definition of a membrane associated polypeptide of a mycobacterium is "unclear" because "a polypeptide can't be capable of detecting an immune response." Applicants respectfully disagree.

Applicants submit that the definition of membrane associated polypeptide as defined is clear. Example 1B (pages 17-19) explains that a colony immunoscreening assay was used to screen genomic *Mycobacterium* libraries against reactivity toward tuberculosis patients' sera. Clones were selected that expressed a protein or protein fragment which specifically reacted with TB sera. Thus, reactive clones identified by this method "detected an immune response" by binding to antibodies of the sera. "Detecting an immune response," therefore, refers to binding of a membrane associated polypeptide to components of TB sera such that a signal can be observed. Applicants submit that a full reading of the specification clarifies the meaning of "detecting an immune response," and therefore, the Examiner's objection to the disclosure should be withdrawn.

Sequence Compliance

The Examiner notes a failure to comply with requirements for patent applications containing nucleotide sequence and/or amino acid sequence disclosures. An amendment is submitted herewith, in a separate paper, which serves to place this application in a condition of adherence to the rules 37 C.F.R. § 1.821-1.825.

Rejection under 35 U.S.C. § 112, first paragraph (Written Description)

The Examiner has rejected claims 24-33 and 41-49 under 35 U.S.C. 112, first paragraph, as containing subject matter not described in the specification in such a way as to

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reasonably convey to one skilled in the art that the inventor had possession of the claimed invention at the time of filing the application. The Examiner states that the specification lacks description of elements essential to the claimed invention, i.e. to the definition of "antigenic determinant," and lacks description of elements essential to the genus antigenic determinant. Additionally, the Examiner states the specification lacks description of elements essential to the genus comprising a homologue of SEQ ID NO:2. Applicants respectfully traverse.

An objective standard for determining compliance with the written description requirement is, "does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed." *See* MPEP § 2163. The fundamental inquiry is whether a claim defines an invention that is clearly conveyed to those skilled in the art at the time the application was filed. *Id.*

The Examiner states that the specification does not describe elements essential to various functions of the claimed invention, including those which are essential to the definition of "antigenic determinant." The Examiner goes on to state that the specification does not describe elements essential to the genus antigenic determinant, what distinguishing attributes are concisely shared by the members of the genus comprising antigenic determinants, and common attributes encompassed by antigenic determinants. Applicants submit that "antigenic determinant" of SEQ ID NO:2 has sufficient support in the specification and in the art to satisfy the written description requirement.

Applicants note that SEQ ID NO:2 was identified based on immunoreactivity against TB sera of the amino acid encoded by SEQ ID NO:2 (Example I). Therefore, SEQ ID NO:2 encodes an amino acid having antigenic determinants relevant to tuberculosis disease. The level of skill in the art for identifying antigenic determinants of given proteins was high at the time the application was filed, in that predicting and identifying such determinants was routine. Accordingly, the skilled artisan could readily identify one or more antigenic determinants in the protein encoded by SEQ ID NO:2. Additionally, the specification states, "The antigens identified here can be further used to determine which segments of these antigens are recognized by Mycobacterium tuberculosis specific T-cells." (Page 29, lines 14-

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17). Thus, the specification itself satisfies the requirements of 35 U.S.C. § 112, first paragraph.

The Examiner states that the specification does not describe the elements which are essential to the genus comprising a homolog of SEQ ID NO:2, nor the distinguishing or common attributes comprising the genus, "homologs of SEQ ID NO:2". Homology of the protein of SEQ ID NO:2 to other proteins is described at page 23, line 6 to page 25, line 40. Specifically, the protein of SEQ ID NO:2 shows closest homology to ion transporting ATPases. Furthermore, the specification states that the protein of SEQ ID NO:2 contains highly conserved residues present in the E1E2-ATPase family (See page 25, line 41 to page 25, line 7). Computer algorithm analyses of SEQ ID NO:2 demonstrate it has a multiple-membrane spanning structure, "in accordance with the channel transport functions of ion-motive ATPases." (See page 26, line 24 to page 27, line 13). Finally, the hydropathy profile of SEQ ID NO:2 was "nearly superimposable" over that of another ATPase. (See page 27, lines 28-29). Thus, "a homolog thereof" would be clearly understood by one of ordinary skill in the art as a Mycobacterial ion transporting ATPase. Applicants submit that the specification and skill in the art fully support the elements "homolog thereof" and "antigenic determinant thereof," and respectfully request that the rejection be withdrawn.

Rejection under 35 U.S.C. § 112, first paragraph (Enablement)

Claims 24-33 and 41-49 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of detecting the presence of antibodies to *Mycobacterium bovis* and *M. tuberculosis*, does not reasonably provide enablement for a method of detecting the presence of antibodies to all Mycobacteria. Applicants respectfully traverse.

Applicants submit that one of ordinary skill in the art, upon reading the specification, would have a reasonable expectation of practicing all of the claimed subject matter. The specification explains that tuberculosis is caused by a variety of species of mycobacteria, including *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti* (page 2, lines 1-2), *M. avium*, *M. intracellulare* and *M. scrofulaceum* (page 2, lines 11-12). SEQ ID NO:2 was obtained by detecting its immunoreactivity with pooled human sera from patients with active pulmonary

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tuberculosis (page 17, lines 16-18). The observed cross-reactivity of SEQ ID NO:2 with pooled anti-sera and the cross-hybridization between SEQ ID NO:2 and DNA of *M. tuberculosis* support the Applicants' position that an immune response of a TB-infected patient can be detected by combining a biological sample, such as patients' sera, with SEQ ID NO:2, a homolog of SEQ ID NO:2, or an antigenic determinant of SEQ ID NO:2. (See page 11, lines 7-20). Furthermore, the protein of SEQ ID NO:2 did not react with sera from normal individuals, nor did it react with other mycobacterial antibodies (See Figure 3 and p.20, lines 8-19).

Applicants also point out that homologs of SEQ ID NO:2 were absent from avirulent forms of Mycobacterium (*M. vaccae* and *M. smegmatis*), supporting a reasonable conclusion that the protein is found in pathogenic forms of Mycobacterium, but not in non-pathogenic forms. (See page 28, lines 24-27; page 30, lines 9-13). Based on this, one of skill in the art would reasonably conclude that the virulent mycobacteria *M. leprae*, *M. africanum*, *M. microti*, *M. avium*, *M. intracellulare* and *M. scrofulaceum* each encode a homolog of SEQ ID NO:2, and that the homolog can react with antibodies produced against SEQ ID NO:2 to detect an immune response to infection by any of these species of mycobacteria.

Additionally, because the sera was pre-incubated with E.coli, it did not cross react significantly with E.coli (see Figure 1 and Example 1). The TB sera used for immunoscreening was absorbed onto E.coli to yield a high signal to noise ratio and eliminate cross-reactive antibodies to E.coli components. Absorption with E.coli eliminated antibodies to mycobacterial antigens with closely related homologs in E.coli, while antibodies to mycobacterial components without E.coli homologs remained in the serum. It is reasonable to conclude that the cloned antigens represent determinants specific to pathogenic or virulent mycobacteria..

Thus, Applicants submit that the claims are in proper form for allowance and request that the rejection be withdrawn.

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Applicants submit that the claims are in form for allowance. If the Examiner believes there are any remaining issues that may be addressed by telephone, she is requested to contact the undersigned attorney at (415) 781-1989.

Respectfully submitted,

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MARKED UP VERSION TO SHOW CHANGES MADE

24. A method of detecting the presence of antibodies to virulent Mycobacterium in a biological sample, said method comprising:
combining said sample with a protein having the amino acid sequence of SEQ ID NO:2, a homolog thereof or an antigenic determinant thereof; and
detecting antibodies bound to said protein.
25. The method of Claim 24, wherein said virulent Mycobacterium is selected from the group consisting of *M. bovis*, *M. tuberculosis*, *M. leprae*, *M. africanum*, *M. microti*, *M. avium*, *M. intracellulare* and *M. scrofulaceum*.

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APPENDIX: PENDING CLAIMS

24. A method of detecting the presence of antibodies to virulent Mycobacterium in a biological sample, said method comprising:
combining said sample with a protein having the amino acid sequence of SEQ ID NO:2, a homolog thereof or an antigenic determinant thereof; and
detecting antibodies bound to said protein.
25. The method of Claim 24, wherein said virulent Mycobacterium is selected from the group consisting of *M. bovis*, *M. tuberculosis*, *M. leprae*, *M. africanum*, *M. microti*, *M. avium*, *M. intracellulare* and *M. scrofulaceum*.
26. The method of Claim 24, wherein said protein is immobilized on a solid support.
27. The method of Claim 26, wherein said solid support is nitrocellulose.
28. The method of Claim 24, wherein said sample comprises one or more of sputum, blood, and serum.
29. The method of Claim 24, wherein said detecting is by a qualitative detection system.
30. The method of Claim 29, wherein said qualitative detection system is a horseradish peroxidase-protein A detection system.
31. The method of Claim 24, wherein said detecting is by a quantitative detection system.
32. The method of Claim 31, wherein said quantitative detection system is a radioimmunoassay.
33. The method of Claim 24, further comprising:
combining a control biological sample with said protein; and
comparing the detection of said binding to the binding of antibodies in the control sample with said protein.

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41. A method of detecting the presence of Mycobacterium in a biological sample, said method comprising:
 - lysing the cells in said sample;
 - combining said lysate with antibodies to a protein having the amino acid sequence of SEQ ID NO:2 or an antigenic determinant thereof; and
 - detecting said antibodies bound to protein in said lysate.
42. The method of Claim 41, wherein said Mycobacterium is selected from the group consisting of *M. bovis*, *M. tuberculosis*, *M. leprae*, *M. africanum*, *M. microti*, *M. avium*, *M. intracellulare* and *M. scrofulaceum*.
43. The method of Claim 41, wherein said lysate is immobilized on a solid support.
44. The method of Claim 43, wherein said solid support is nitrocellulose.
45. The method of Claim 41, wherein said detecting is by a qualitative detection system.
46. The method of Claim 45, wherein said qualitative detection system is a horseradish peroxidase-protein A detection system.
47. The method of Claim 41, wherein said detecting is by a quantitative detection system.
48. The method of Claim 47, wherein said quantitative detection system is a radioimmunoassay.
49. The method of Claim 41, further comprising:
 - culturing a diagnostic sample to produce colonies of bacteria present therein, whereby said culture represents said biological sample.
50. (New) A method of detecting the presence of antibodies to a virulent Mycobacterium in a biological sample, said method comprising:
 - combining said sample with a purified protein of a mycobacterium other than *M. bovis* BCG, wherein said protein
 - is a homolog of the protein of SEQ ID NO:2;
 - is an immunogenic membrane-associated protein of said mycobacterium; and
 - is encoded by DNA which is capable of hybridizing with a DNA probe having the complete sequence represented in SEQ ID NO: 1 under conditions where, on a Southern blot, said probe will identify single 3.25 kb BamHI fragments from *M. bovis* BCG and *M. tuberculosis* H37Rv DNA, but will not hybridize with BamHI-digested DNA from either *M. smegmatis* or *M. vaccae*.